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Special Focus Report

Early gene changes induced by isotretinoin in the skin provide clues to its mechanism of action

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Gene expression analysis of patient skin at one-week of oral isotretinoin therapy for severe acne illustrates the initial changes caused by 13-*cis* RA. Focus on the function of these significantly changed genes provides clues to its mechanism of action in skin. Significant gene changes included the upregulation of *lipocalin 2*, which encodes the protein neutrophil gelatinase associated lipocalin (NGAL). Detailed studies of NGAL's localization and function within the sebaceous gland demonstrated that NGAL mediates the apoptotic action of 13-*cis* RA on sebaceous glands, thereby suggesting that agents which selectively induce NGAL expression in sebaceous glands might represent therapeutic alternatives to the use of 13-*cis* RA for the treatment of acne.[1]

In addition to NGAL, 13-*cis* RA affected the expression of numerous other genes in patient skin at one-week and in our sebocyte cell culture model, SEB-1. The results of these gene expression array analyses have been submitted to the National Center for Biotechnology Information Gene Expression Omnibus Database and are available under series accession number GSE10434. These early gene changes can be broadly categorized as tumor suppressors, protein processors and genes involved in transfer or binding of ions, amino acids, lipids or retinoids. Investigating the function of these potential candidate genes that mediate retinoid response in the skin provides a better understanding of the complete actions of 13-*cis* RA within skin with the goal of discovering safer alternatives to oral retinoid use in the treatment of acne and other skin diseases. For example, tazarotene induced gene 1 [*TIG1*, retinoic acid responder 1 (*RARRES1*)] encodes a tumor suppressor belonging to the latexin family of proteins, whose promoter is methylated (CpG island) and therefore silenced in a variety of cancers.[2-5] The increase in expression of *TIG1* induced by 13-*cis* RA may mediate the known effects of this drug in chemoprevention of skin cancer in addition to the known suppressive effects on sebocyte proliferation.[6-9] Furthermore, genes encoding both serine proteases and serine protease inhibitors were upregulated by 13-*cis* RA at one-week. Serine protease inhibitors (serpins) are involved in tissue remodeling and control of inflammation.[10] Increased expression of serpins have been reported in inflammatory processes such as psoriasis and inflammatory acne lesions.[11,12] Since 13-*cis* RA is the most potent agent available to reduce severe inflammatory acne, it is possible that the upregulation of serpins, which in turn, scavenge pro-inflammatory proteins, mediates the anti-inflammatory effect of 13-*cis* RA. In support of this hypothesis, previous HPLC studies demonstrated that 13-*cis* RA can be isomerized to ATRA within sebocytes[9] and it has been shown that SERPINA5 is capable of binding ATRA in vitro and may function in retinoid transport.[13,14]

13-*cis* RA significantly increased expression of multiple members of the solute carrier family of proteins. Recent data have highlighted the importance of the solute carrier family of proteins in skin biology. For example, *SLC12A8*, which is upregulated within our one-week analysis, encodes for a sodium/potassium/chloride transporter that has recently been identified as a candidate gene for psoriasis susceptibility and is contained within the PSORS5 locus of CHR3q.[15,16] Although, no mutations in *SLC12A8* have been linked to psoriasis, it is interesting to note that it is retinoid responsive. *ATP12A*, another significantly upregulated gene at one-week, encodes sodium/potassium ATPase, an integral membrane protein involved in solute transport responsible for the hydrolysis of ATP coupled with the exchange of hydrogen and potassium ions across membranes. This particular protein regulates ion flux into melanosomes and affects tyrosinase activity.[17] Interestingly, another cation transporter (SLC24A5) has been identified as playing a prominent role in skin biology by regulating flux across the melanosome membrane in zebrafish and this gene links to skin color in humans.[18] No other studies to date have examined the other solute carrier molecules and their relationship to normal human skin or skin disorders.

Retinoids are crucial to epidermal development and differentiation.[19] S100 proteins modulate cellular differentiation, energy metabolism, cytoskeletal membrane interactions and cell cycle progression.[20] S100 protein family members are upregulated in our one-week patient array as well as our SEB-1 sebocytes after 13-*cis* RA treatment. Interestingly, the upregulated S100 proteins are induced by oxidative or inflammatory stress and *S100A7* (psoriasin) may function as a chemo-attractant agent for immune cells.[20] One can speculate that initial upregulation of S100 proteins by 13-*cis* RA is responsible for the "acne-flare" response observed in some patients receiving oral or topical retinoids for the treatment of their acne.

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Approximately half of the significantly changed genes affected by 13-*cis* RA contained consensus sequences for RAR or RXR receptors. This study does not address the question of whether the changes in gene expression are the direct result of 13-*cis* RA, one of its metabolites or if retinoid receptors are involved. Instead, attention is focused on the function of these significantly changed genes and how these functions may provide clues to 13-*cis* RA within the sebaceous glands and whole skin.

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